Let us consider the differential form of equation (1), i.e.,

$$\frac{dx}{dt} = K\,(x + b)\,(A - x) \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

If we let $x = x' - b, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$

then $dx = dx' \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$

and on substituting (4) and (5) in (3) we get

$$\frac{dx'}{dt} = K\,(x')\,[(A + b) - x'] \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Thus, by a simple linear transformation, we may obtain a differential equation which is precisely of the same type as the differential form of equation (2), that is, it is of the same type as

$$\frac{dx}{dt} = K\,(x)\,(A - x) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Since the curve of equation (2) is symmetric, it follows that the curve of equation (1) is also symmetric and that the two halves of the cycle on either side of the moment of maximum growth velocity are *not* unequal in slope and amplitude.

That the curve of equation (1), or (1′), is symmetric may be demonstrated, in another manner, as follows:

In the case of the equation,

$$\ln \frac{x + b}{A - x} = k\,(t - t_1) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1')$$

it may be shown readily that the point of inflection is at $\left[(t_1), \left(\frac{A - b}{2}\right)\right]$. Now, if equal increments of abscissa (t) to the right and to the left of t_1 define values of x which are equidistant from the ordinate $\left(\frac{A - b}{2}\right)$, the curve is symmetric about the point of inflection. Such is the case, for when

$$t = t_1 + n,^{9} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

$$x = \frac{A\,\epsilon^{nk} - b}{1 + \epsilon^{nk}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

[9] n being any real number.

and when $$t = t_1 - n, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

$$x = \frac{A - b\,\epsilon^{nk}}{1 + \epsilon^{nk}}, \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

and the distance between the ordinates of the curve at

$$\left[(t_1 + n), \left(\frac{A\,\epsilon^{nk} - b}{1 + \epsilon^{nk}}\right)\right] \text{ and at } \left[(t_1), \left(\frac{A - b}{2}\right)\right] \text{ is}$$

$$\frac{A\,\epsilon^{nk} - b}{1 + \epsilon^{nk}} - \frac{A - b}{2} \text{ or } \frac{(A + b)\,(\epsilon^{nk} - 1)}{2\,(1 + \epsilon^{nk})} \quad \ldots\ldots\ldots (\alpha)$$

and the distance between the ordinates of the curve at

$$\left[(t_1), \left(\frac{A - b}{2}\right)\right] \text{ and at } \left[(t_1 - n), \left(\frac{A - b\,\epsilon^{nk}}{1 + \epsilon^{nk}}\right)\right] \text{ is}$$

$$\frac{A - b}{2} - \frac{A - b\,\epsilon^{nk}}{1 + \epsilon^{nk}} \text{ or } \frac{(A + b)\,(\epsilon^{nk} - 1)}{2\,(1 + \epsilon^{nk})} \quad \ldots\ldots\ldots (\beta)$$

The quantities (α) and (β) are identical and thus it has been demonstrated that the curve is symmetric, since equal increments, to the right and to the left of t_1, define values of x which are equidistant from the ordinate $\left(\frac{A - b}{2}\right)$.

By suitable algebraic treatment we may also demonstrate, independently of the above, that the slope of the two branches of the curve is the same. If we substitute the ordinates $\left(\frac{A - b}{2} + m\right)$[10] and $\left(\frac{A - b}{2} - m\right)$ in the equation for the slope of the curve defined by equation (1), that is, in

$$\frac{dx}{dt} = \frac{k}{A + b}(x + b)(A - x) \quad \ldots\ldots\ldots\ldots (12)$$

we get, in the first case:

$$\frac{dx}{dt} = \frac{k}{A + b}\left(\frac{A - b}{2} + m + b\right)\left(A - \frac{A - b}{2} - m\right)$$

$$= \frac{k}{A + b}\left[\left(\frac{A + b}{2}\right)^2 - m^2\right], \quad \ldots\ldots\ldots\ldots (\gamma)$$

[10] m being any real number.

and in the second case:

$$\frac{dx}{dt} = \frac{k}{A + b}\left(\frac{A - b}{2} - m + b\right)\left(A - \frac{A - b}{2} + m\right)$$

$$= \frac{k}{A + b}\left[\left(\frac{A + b}{2}\right)^2 - m^2\right], \ldots\ldots\ldots\ldots\ldots (\delta)$$

The expressions (γ) and (δ) are identical and thus it has been shown that the slope of the curve at any two points, which are equidistant from the point of inflection, is the same; and hence the slope of the two branches of the curve is the same.

Summary

Several incorrect statements regarding the curve defined by the autocatalytic equation,

$$\ln\frac{x + b}{A - x} = K(A + b)(t - t_1), \text{ or } \ln\frac{x + b}{A - x} = k(t - t_1)$$

have been made in the literature. These statements are to the effect that:

(1) the curve described by the above equations is asymmetric, and

(2) the two halves of the curve have unequal slopes.

It has been demonstrated in this paper that these statements are incorrect.

MICROBIOLOGY.—*Myxamoebae in soil and decomposing crop residues.*[1] Charles Thom and Kenneth B. Raper, Bureau of Chemistry and Soils.

Amoebae are regularly observed and reported by students of soil organisms. Sandon in his book on Soil Protozoa and Waksman in his "Principles" reviewed the information available to 1927. Sandon supplemented the literature by summarizing the studies made at the Rothamsted Station. He made no reference to the amoeboid phase of the Myxomycetes and the Acrasieae as members of the soil population with characters sufficiently suggestive of protozoa to open the possibility of confusion. Brierley (1928 p. 16) listed five genera of Myxomycetes as occurring in soil with "evidence that they may live vegetatively in this habitat." Waksman in his "Principles", p. 236 refers to the Myxomycetes as including species which are plant para-

[1] Received June 17, 1930.

sites with the comment that they appear able to maintain themselves independently in the soil. Krzemieniewski reported that by proper culture methods many Myxomycetes may be obtained in culture from the soil. Harper, following Krzemieniewski's method, isolated *Polysphondylium* from soil collected in New York City parks. The extensive cultural studies reported by Olive and others have been primarily concerned with obtaining and identifying the fruiting bodies of this group of organisms. Very little has been reported to indicate the distribution and significance of the amoeboid phase or even the plasmodium phase of these organisms in the soil or in the decaying vegetation of the meadow or the cultivated field. We were surprised, therefore, to encounter these organisms in great numbers in the course of studies begun for entirely other purposes.

In December 1929, samples of decaying grasses and weeds were collected in an experimental field on the Arlington Farm of the United States Department of Agriculture. When brought to the laboratory, selected leaves and stems were cut into convenient lengths and dropped upon the surface of solidified mannite agar in petri dishes to permit certain saprophytic organisms present to develop. The nutrient medium used was free from nitrogen or nearly so, hence considered only as furnishing a moist substratum to favor the further development of organisms already present upon the grass.

Within a week several myxomycete plasmodia developed and moved about upon the agar in these plates. Thousands of amoebae or myxamoebae also spread upon the agar from pieces of decaying grasses and weeds. Masses of bacteria and mold mycelium covered and spread outward from every piece of decaying vegetation. Since we could find no record of observations of Myxomycetes under such conditions several series of such cultures were made to extend our knowledge of the presence and abundance of these forms under winter conditions in Washington and vicinity.

The first of these samples consisted of a few leaves of crab grass collected on February 6th from a roadside. Prior to this, the grass had been covered by snow for several days, and was quite wet when brought to the laboratory. The leaves were cut into convenient lengths and placed upon mannite agar in petri dishes and the dishes were held at room temperature. In the course of a few days, plasmodia were observed in all the plates. Large "amoebae" and small amoeboid cells, possibly myxamoebae, were present in considerable numbers; the latter were particularly numerous. Using a small sterile pipet, a part of one of the plasmodia was transferred on Febru-

ary 12 to a fresh mannite agar plate. To this culture was added from time to time a suspension of dead bacteria belonging to the *B. aerogenes* group. The plasmodium grew slowly but consistently until March 8th when it was transferred to hay infusion agar medium. By frequent transfers it is still in actively growing condition on this medium after four months. After the snow melted, samples of decaying grass were again collected from the field on February 8th. These samples were plated and incubated as before, and again in the course of a week plasmodia developed in all the plates. Two plates contained particularly well developed grayish-white plasmodia. These were more or less fan shape, measuring 4 cm. across the "fan," with stream of protoplasm extending back for several centimeters along the path, which the main portion of the plasmodium had recently traversed. One of these fruited on February 17th, producing about 150 sporangia which belonged to the genus *Didymium*. On February 18th a part of the other plasmodium was transferred to hay infusion agar and is still active in culture in May. The portion not transferred fruited two days later as a *Didymium*.

On February 17 wide mouth bottles of approximately one liter capacity were half filled with wet sand and sterilized. Samples of decaying grass from the field were placed on the sand in a rather compact mat three fourths of an inch in depth. By the end of the first week one of the twelve bottles thus prepared contained a visible plasmodium which climbed up on the side of the bottle. It was grayish-white in color and measured 1.5–2 centimeters in diameter. During the following week plasmodia were observed in two additional bottles. On February 27th, a few leaves from three of the bottles not containing visible plasmodia were dropped on mannite agar. Plasmodia developed in three-fourths of the plates. (See numbers 9, 12 & 13 in table 2). It was evident that the agar medium was not necessary for the development of plasmodia.

On February 20 (a warm period in 1930) the tobacco fields of the Bureau of Plant Industry and the University of Maryland, situated near Marlboro, Maryland, were visited. Various samples were collected. One mass of decaying annual grasses and the soft soil down to about 10 cm. and totalling perhaps 1 liter was placed in a bag and brought to the laboratory where it was transferred to a covered dish about 25 cm. in diameter and 10 cm. deep without adding any water. In approximately one week a plasmodium moved up from this mass out upon the glass and spread over about half of the inside of the

glass cover. During the following night the fruit bodies of a species of *Didymium* were produced.

In other dishes numerous small plasmodia were produced and spread outward from pieces of grass, ragweed stems and stems of *Erigeron* collected from various tobacco plots, and scattered over mannite agar. In some dishes millions of small myxamoebae were seen and later fruited abundantly as *Dictyostelium*.

In these various plates, microscopic examination regularly showed many encysted as well as active myxamoebae. Microscopic mounts from the dry stems and leaves as brought to the laboratory showed many such cysts which appeared to be similar to those which developed from time to time in the cultures. From these observations it was evident that myxomycetes and allied forms are well represented in the tobacco fields of Marlboro.

In the samples collected and plated thus far, no attempt had been made to separate the standing leaves and culms from those lying on the soil. The question now arose as to whether the myxomycetes were present only in the basal portion of standing grass leaves and in leaves lying on the soil, hence protected against extremes of temperature and desiccation, or if they were also present in leaves standing several inches above the soil. And if present in both, what was their relative abundance in the two? To determine this point a series of samples were collected; the uppermost portion of standing leaves and those lying on the soil were collected and plated separately, the former type being designated by "A" following the sample number, the latter type by "B" following the same sample number. The first of these were collected on February 21st; other samples being taken at later dates. Plasmodia appeared in 70% of all plates prepared from "A" samples and in 67.7% of those prepared from "B" samples. A full account of these platings is given in table 1.

During the same period some additional "composite" samples were collected and plated. Plasmodia appeared in 71% of all plates prepared from these samples. A detailed account is given in table 2.

All the myxomycetes thus far isolated from decaying grass, and cultured as above until fruits were produced, belonged to the genus *Didymium*. Species have not been determined.

Steps were then taken to determine whether or not plasmodia could be obtained from the soil underlying the sod from which the grass samples had been taken. Soil samples from varying levels ranging from the surface to a depth of twelve inches were diluted with 10 cc.

of sterile water, and the resulting suspension streaked on mannite agar plates by means of a platinum loop. Four or five streaks, a single drop of the suspension being used for each, were made across a plate and two or three plates prepared for each sample. Twenty-seven

TABLE 1.—GRASS SAMPLES—STANDING AND THAT ADJACENT TO SOIL PLATED SEPARATELY

Sample No.	A. Grass erect		B. Grass on soil		Days after inoculation	Type of grass	Date of sampling	Remarks
	No. of plates	Plates containing plasmodia	No. of plates	Plates containing plasmodia				
2	2	0	2	1	12	Rye	Feb. 21	Rye stems. Last summer's growth
3	4	1	4	2	12	Orchard grass	Feb. 21	Samples 2 to 5 inclusive taken in very warm weather for Feb. Grass very dry
4	4	3	4	3	12	—	Feb. 21	
5	4	2	4	2	12	—	Feb. 21	
15	5	5	4	3	9	Bluegrass	Feb. 27	Samples 15 & 16 taken day after rain. Grass moist. Temperature high (Feb.)
16	4	4	4	4	9	Orchard grass	Feb. 27	
18	4	0	4	1	12	Bluegrass	Mar. 20	See #19 below
22[a]	2	2	2	2	14	Sedge	Mar. 22	Samples dry
23	3	3	3	3	14	Velvet grass	Mar. 22	Temperature rather low but not freezing
24	4	1	4	2	14	Orchard grass	Mar. 22	
25[b]	3	3	3	3	17	Bluegrass	Mar. 22	
26	5	5	5	3	17	Bluegrass	Apr. 11	Samples 26–29 inclusive. Taken following heavy rains. Temperature 60–70°
27	3	3	3	2	17	Velvet grass	Apr. 11	
28[b]	5	5	6	5	17	Bluegrass	Apr. 11	
29	4	2	3	0	17	Orchard grass	Apr. 11	
19	4	3	4	4	12	Velvet grass	Mar. 20	Samples 18 & 19 rather dry. Temperature 40–55°F.
	60	42	59	40				
	= 70%		= 67.7%					

[a] Sedge collected from west end of reservoir, sample dry when taken but had been covered with water during much of winter.

[b] Very heavy sod in each case.

samples from five "borings" were collected. The plates were examined after incubation at room temperature from ten days to two weeks. Amoeboids, amoebae or myxamoebae were found to be very numerous in all the samples, even those taken from a depth of twelve inches, but plasmodia developed from only two of the twenty seven samples.

One was obtained from a sample of earth collected at a depth of four inches underlying a blue grass sod on March 11th, and resembled closely the plasmodia of *Didymium* which we have in culture. The other was obtained about two weeks later from a sample taken at a depth of three inches underlying another blue grass sod in the same

TABLE 2.—Grass Samples (A. & B. Not Separated)

Sample No.	A. & B.		Number days after sampling	Date of sampling	Type of Grass	Remarks
	No. of plates	Plates containing Plasmodia				
1	6	5	12	Feb. 21	Rye-Compost heap	Rye cut last summer and thrown on compost heap. Samples taken from this heap
9	4	2	8	Feb. 25	Orchard and Bluegrass	Samples 9 and 10: Grass from Walker Hill placed in bottles
10	3	0	8	Feb. 25	" "	half filled with sand 2–17–30. Set in greenhouse. Samples from these plated
M8	6	4	12	Mar. 3	—	Grass collected from experiment station at Marlboro
12	2	2	9	Feb. 27	Orchard and Bluegrass	Samples 12 to 14 inclusive taken
13	2	2	9	Feb. 27	" "	from bottles in greenhouse
14	4	3	9	Feb. 27	" "	prepared as #9 & 10
17	4	3	14	Feb. 27	—	Sample taken day after rain. Temperature high for February
20	4	3	12	Mar. 20	Orchard grass	Grass rather dry. Temperature 40–55°F.
30	4	4	14	Apr. 23	—	Rather dry grass collected from roadside at reservoir, Arlington Farm
31[a]	6	4	14	Apr. 23	Sedge	Sedge collected from west end of reservoir. Samples dry
	45	32				
	= 71%					

[a] This sample gave heavy growth of azotobacter in all plates. Nematodes were more numerous than in other samples.

field. This plasmodium was not isolated; but in the original plate it resembled rather closely plasmodia of *Stemonitis*, which had been grown from spores in this laboratory for comparison. This *Stemonitis* plasmodium consisted of very close networks of delicate strands of colorless protoplasm.

Dictyostelium was obtained in similar cultures made from two plots of Leonardtown loam under experimental study in the greenhouse including one plot limed to pH 7.1 and the other with about pH 4.2. Cultures from six other plots in the greenhouse showed abundant amoeboids, but no myxomycete was positively identified.

Among the other experiments already performed, the effect of seasonal changes in temperature have been rather striking. The plasmodia in culture were not much affected by small changes of temperature, but have shown decided dislike for temperatures above 20–22°C. This was particularly evident during the latter part of April and the early part of May, when there was extremely warm weather. The plasmodia growing in the laboratory prior to this became less active and grew very slowly. During the following week this effect was even more pronounced. Plasmodia in most cases broke up into sclerotia and in many cases disintegrated quite completely. Those that were still viable were placed in an incubator with a temperature range of from 15 to 18°C. Within a very short time normal growth and activity were resumed. Subsequent culture experiments have been carried at both incubation temperatures with the forms in culture showing decided preference for the cooler condition.

In another series of studies, dilution cultures at 1 to 50, 1 to 500 and 1 to 5,000 were made to test the presence of protozoa in plots of land containing decomposing rye and vetch. In certain of these cultures, amoeboid organisms were predominant. Mannite plates were streaked from these tubes. Of 15 such cultures, one produced *Dictyostelium* and another produced plasmodia.

These observations are recorded to call attention to the presence of myxomycete amoeboids as part of the soil population. Experiments in culture of these forms and efforts to determine their function as part of such populations are in progress. Meanwhile search of the literature furnished little information on the occurrence of Myxomycetes in the soil, and no direct reference to the isolation of plasmodia from decaying vegetation under winter conditions such as described in this paper. The following references are worthy of note: Miller (6) working in the Johns Hopkins Medical laboratories obtained *Stemonitis* plasmodia as contaminants in protozoa cultures which were being grown in tap water to which had been added unsterilized hay. He then collected hay from various sources and again plasmodia were obtained. He expresses the opinion that "plasmodia are constantly present on hay in one form or another." Lister (5) in his monograph of the Mycetozoa lists dead leaves and twigs as the most